REMARKS/ARGUMENTS

The Pending Claims

Claims 1, 7-14, and 17-26 are pending. Pursuant to a restriction requirement, claims 7-14 and claims 21-26 have been withdrawn. Therefore, claims 1 and 17-20 are currently subject to examination.

Claim Amendments

Claims 1 and 20 have been amended to replace "mammal" with "macaque or human." Support for this amendment is found throughout the specification as originally filed, *e.g.*, at paragraph [0010] and Example 2. No new matter has been added by way of these amendments.

Discussion of the Enablement Rejection

Claims 1 and 17-20 remain rejected under Section 112, first paragraph, as allegedly failing to comply with the enablement requirement. The rejection is respectfully traversed.

The model system study of the Examples shows upregulation of Cripto-1/SEQ ID NO: 1 is indicative of the presence of NeuroAIDS. However, in the most recent Office Action, the USPTO alleges that (i) the study only used a single control and, essentially, the study cannot be used to determine indication of a disease due to a small control sample size, (ii) post-exsanguination collection of tissue cannot be used to reliably determine gene expression, (iii) the model system detected the macaque homologue of the human sequence (SEQ ID NO: 1) of Cripto-1 in macaque NeuroAIDS and would not indicate upregulation of human Cripto-1 in human NeuroAIDS, (iv) there were no studies performed in humans, (v) the macaque monkey is not a reasonable model for humans and SHIV infection is not a reasonable model for NeuroAIDS, (vi) there is no nexus for the role of increased SEQ ID NO: 1 and NeuroAIDS, (vii) Raghaven et al., Brain Pathol., 7:851-861 (1997) ("Raghaven") teaches SHIV has different affects on two members of the macaque family and that SIV- and SHIV-induced NeuroAIDS have different pathologies, and (viii) Enard et al., Science, 296:340-343 (2002) ("Enard") teaches intra- and interspecies variation in gene expression in brain tissue is substantial, and Cheung et al., Nat. Genet., 33:422-425 (2003) ("Cheung")

teaches there is natural variation in gene expression among different individuals and renders unpredictable any correlation between altered gene expression and phenotype.

Moreover, in a previous Office Action, the USPTO alleged that (ix) knowledge of the particular housekeeping genes used in a gene expression study is required, (x) Vandesompele et al., Genome Biol., 3:1-11 (2002) ("Vandesompele") teaches at least three housekeeping genes are required for accurate normalization of gene expression data and that using one gene for normalization is not reliable due to large variation in housekeeping gene expression, (xi) Wu, J. Pathol., 195: 53-65 (2001) ("Wu") teaches that conclusions drawn from gene expression data depend heavily on the particular choice of data analysis, and (xii) Newton et al., J. Comput. Biol., 8:37-52 (2001) ("Newton") teaches that a basic statistical problem is determining when the measured differential expression is likely to reflect a real biological shift in gene expression, and replication of data is critical to validation.

In response to the previous Office Action, Applicants filed a response on March 4, 2009 including a Declaration of Dr. Nancy E. J. Berman ("the Berman declaration") under 37 CFR 132, a co-inventor on the instant application.

The Examiner has stated in the most recent Office Action (i.e., after Applicants' response filed March 4, 2009) that the arguments based on Wu, Newton and Vandesompele have been withdrawn (page 18, first full paragraph). The arguments based on Wu, Newton and Vandesompele correspond to issues xi, xii, and x above. Also, since this Office Action has not repeated the previous arguments based on the housekeeping genes, it is believed that the Examiner's arguments regarding this issue (issue xi above) have been withdrawn as well. Nevertheless, it appears that the Examiner has maintained the rejection based on Wu (page 8, final paragraph). Therefore, the allegations regarding (i) through (viii) and (xi) are traversed and are addressed below.

(i) Controls: Sample Size and Infection

The Examiner alleges that the study of the instant specification only used a single macaque control and, essentially, the study cannot be used to determine indication of a disease due to a small control sample size. The Examiner also states that the control was not infected by any virus and thus alleges that expression of the macaque Cripto-1 observed may

be a result of viral infection generally, *i.e.*, not specific to HIV/SHIV infection (pages 10-11, bridging paragraph).

The Manual of Patent Examining Procedure ("M.P.E.P.") makes clear that an analysis of enablement involves considering what one of ordinary skill in the art would have known at the time of filing the patent application at issue:

> 35 U.S.C. 112 requires the specification to be enabling only to a person "skilled in the art to which it pertains, or with which it is most nearly connected." In general, the pertinent art should be defined in terms of the problem to be solved rather than in terms of the technology area, industry, trade, etc. for which the invention is used.

M.P.E.P. at § 2164.05(a). For the instant invention, the pertinent art would have related to a method of detection of the expression level of a particular sequence (*i.e.*, SEQ ID NO: 1) in the central nervous system ("CNS") to indicate the presence of NeuroAIDS. Thus, the problem to be solved, as was done by the inventors of the instant application, was to provide a reliable method of indicating the presence of NeuroAIDS. Therefore, one of ordinary skill in this art trying to solve this problem would have been concerned with using methods recognized in the art to study the NeuroAIDS disease in determining methods of indicating the presence of NeuroAIDS.

As described previously, one of ordinary skill in the art of studying NeuroAIDS would have recognized that the macaque monkey animal model was an acceptable animal model for studying NeuroAIDS in humans (Berman declaration at ¶ 10), that such animal models overcame obstacles of performing NeuroAIDS studies in humans (Berman declaration at ¶ 9), and that macaque animal models were necessarily performed on a small scale (Berman declaration at ¶ 6). Some macaque NeuroAIDS studies (such as that most recently cited by the Examiner in Roberts, et al., Am. J. Pathol., 162:2041-2057 (2003) ("<u>Roberts</u>")) have used more than one control animal. However, studies using only one control would have been acceptable to one of ordinary skill in the art, as Dr. Berman asserts in the Berman declaration. Indeed, as discussed in Berman declaration at ¶ 10, the studies described in the Examples of the specification of the instant application, using a single control animal, have been published in Stephens et al., Neurosci. Lett., 410:94-99 (2006) ("<u>Stephens</u>").

The Examiner contends, "[P]ublication in a peer reviewed journal does not demonstrate the study or method used in the study is patentable" (page 15, first full paragraph). However, Applicants submitted the publication as evidence of the acceptability of the method to those of ordinary skill in the art who reviewed the article as peer reviewers prior to its publication. This, then, is probative of whether other ordinarily skilled artisans would have thought a study using a single control animal would have been sufficient to reliably report on upregulation of genes in a macaque animal model of NeuroAIDS. Since the peer reviewers of Stephens allowed the study to publish without requiring additional controls be shown, this demonstrates that the state of the art at the time of publication was such that one control animal in a macaque animal study was acceptable to those in the pertinent art – which, here, as discussed above, is to provide a reliable method of indicating the presence of NeuroAIDS. The Examiner additionally cites Cheung as showing there are variations in individuals of species, that this makes use of a single control animal unreliable in that any results would be unpredictable, and that the results based on a single control animal may be statistical outliers. As above, the analysis of enablement relates to what one of ordinary skill in the art would have recognized at the time of the filing of the instant application. Since Stephens was published in 2006 and describes the studies performed in the instant application, this demonstrates that a single control animal was acceptable to ordinary artisans (*e.g.*, the peer reviewers) in this field of art even years after the filing of the instant application (priority date of 2003) and publication of Roberts (published in 2003) and Cheung (published in 2003).

An ordinary artisan in the field of pertinent art, as discussed above, at the time of filing of the instant application would have also recognized that control animals would not have been infected with any virus at all. The Examiner appears to contend that the infective act of a virus alone may induce gene expression. Applicants take no position on whether certain genes are upregulated due to this and need not do so. At issue is what one of ordinary skill in the pertinent art would have recognized as acceptable to determine whether upregulation was due to a particular virus or disease-state, here NeuroAIDS. Indeed, Roberts teaches that all control macaques were uninfected (Roberts at page 2042, second column, first full paragraph), even the ones that received the positive control CD8+ cell-depleting regimen; yet Roberts also reports on genes that were upregulated (Roberts at Table 2 on pages 2044-

2045). Therefore, Roberts supports the position that control animals would not be infected with any virus at all.

Finally, the Examiner has stated that Roberts does not show the upregulation of SEQ ID NO: 1 (Cripto-1) even though it is a macaque NeuroAIDS study (pages 7-8, bridging paragraph), as well as stating that Buch et al., J. Neuroimmunol., 157:71-80 (2004) ("Buch") does not show upregulation of Cripto-1. At the outset, Applicants note that the Examiner has withdrawn most of the arguments regarding detection (outlined as (ix) though (xii) above) that are pertinent to microarrays (*e.g.*, as used in the instant application), presumably in order to cite Roberts since Roberts uses microarray techniques to show upregulation of genes and since Roberts does not follow all of the procedures the Examiner previously argued were required for microarray data analysis. Therefore, since the Examiner has dropped these arguments, and additionally in view of the arguments below, Applicants have demonstrated that the microarray studies used in the specification as originally filed were an effective way to analyze upregulation of Cripto-1.

Additionally, one of ordinary skill in the pertinent arts would have recognized that the claims of the instant application were enabled at the time of filing since the laboratory which published Roberts actually supported the methods used by the Applicants. Roberts is a paper authored by several authors from The Scripps Research Institute, including Howard S. Fox. Prof. Fox also published a paper only nine months prior to Roberts entitled, "Analysis of Result Variability from High-Density Oligonucleotide Arrays Comparing Same-Species and Cross-Species Hybridizations" (Chismar et al. (including H. S. Fox), Biotechniques, 33:516-8, 520, 522 passim. (2002), "Chismar"). Chismar is a microarray study that used the same Affymetrix human U95av2 GeneChip microarray as Roberts (Chismar at pages 518-7, bridging paragraph and Roberts at page 2042, second column, final paragraph); and Chismar compares variability in cross-species hybridization experiments, specifically between rhesus macaques and humans. First, as will be discussed in more detail below, Chismar (and thus the laboratory of Roberts), at around the time of filing the instant application (2002-2003), showed the use of macaque models to report on human health and disease was accepted by one of ordinary skill in the art, even with variability among species. Thus, the upregulation of macaque Cripto-1 in macaque NeuroAIDS would reliably report on the upregulation of human Cripto-1 (SEQ ID NO: 1) in human NeuroAIDS. Second, Chismar shows that, even

though cross-species hybridization is an acceptable method, some genechips will have probes that do not hybridize well and that more detailed studies or confirmation may be helpful, including using PCR (page 518, first column, first full paragraph). Applicants used a different microarray than Chismar/Roberts, one from Clontech, not Affymetrix (specification as originally filed at paragraph [0073]). Therefore, based on the teachings of Chismar, the Affymetrix microarray of Roberts may have had probes to Cripto-1 which would not hybridize as well to macaque Cripto-1 as the probes in the Applicants' Clontech microarray. Regardless, and more importantly, Chismar advocates that there should be confirmation of the microarray studies, *e.g.*, by PCR studies, especially if there is no upregulation determined (Chismar at page 518, first column, first full paragraph, penultimate and final sentences), as was the case in Roberts when Cripto-1 was found not to be upregulated in the Affymetrix array. However, Roberts only confirmed the upregulation of 16 upregulated genes observed in the Roberts Affymetrix microarray by immunohistochemistry and *in situ* hybridization (Roberts at page 2043, first column, second paragraph) and did not perform confirmation studies on any "negative"/non-upregulated genes. Therefore, it is unknown whether the non-observance of upregulation of Cripto-1 in Roberts was due to a false negative as cautioned in Chismar. Since Chismar and Roberts are from the same laboratory, the Fox lab may be focused on the genes upregulated in the preliminary Affymetrix microarray experiments since those were easily identified – as testing those that were not upregulated would be more laborious. However, it was through the discovery of the Applicants that it was found Cripto-1 is upregulated during NeuroAIDS. Not only did the Clontech microarray identify Cripto-1 as upregulated, the Applicants additionally confirmed Cripto-1 upregulation using RT-PCR (specification as originally filed at, *e.g.*, paragraphs [0075] and [0076]), as advocated by Chismar. Therefore, one of ordinary skill in the art would have recognized that the Applicants performed all of the recognized steps to determine upregulation of a particular gene and that Cripto-1 is indeed upregulated even though Roberts does not show Cripto-1 upregulation. Indeed, the lab group which published Roberts even admits that no observance of upregulation may be a false negative, which needs further investigation and confirmation. It was the Applicants who provided that further investigation *and* confirmation to conclusively show upregulation of Cripto-1 during NeuroAIDS.

As for Buch, this paper is a review article of the methods and results of in the art (*see* abstract, final sentence). Buch cites Sui et al., J. Med. Primatol., 32:229-239 (2003) ("Sui")

as showing upregulation of certain genes in macaque/SHIV animals but not Cripto-1 based on the Clontech array used in the instant application (Buch at page 73, first column, final paragraph and Sui at page 231, first column, first full paragraph). Buch then cites that upregulated genes were determined using an Affymetrix array but does not identify Cripto-1 (Buch at *Id.* and page 74, Table 1). However, the above arguments apply here as well. First, Sui did not confirm up/down-regulation since they focused on leukocyte interferon-inducible peptide (IP-10), interleukin-4 (IL-4), interleukin-7 (IL-7) platelet-derived growth factor (PDGF)-B chain, brain-derived neurotrophic factor (BDNF), neurotrophic factor 4 (NT4), and β-actin as a control (Sui at pages 231-232, bridging paragraph), but not Cripto-1. Also, the Affymetrix array may have differences that would not necessarily detect Cripto-1 upregulation. Therefore, since Buch was published in 2004 after Roberts and Sui were published in 2003 (and Roberts and Sui (incorrectly) did not show upregulation of Cripto-1), but published before Stephens in 2006 (which did show upregulation of Cripto-1 based on art acceptable methods *and* confirmation), it is not surprising that Buch (as well as Sui) does not show upregulation of Cripto-1. The value of Buch and Sui are that they show, as discussed more below, that macaque NeuroAIDS was an acceptable method of indicating conditions in human NeuroAIDS (as long as confirmatory methods were performed as in Chismar, which were done by the Applicants). It was the Applicants who did perform the studies of Roberts/Buch/Sui *and* the confirmation Chismar for Cripto-1.

(ii) Exsanguinations

The Examiner continues to allege that post-exsanguination collection of tissue cannot be used to reliably determine gene expression. The Examiner also asserts that the Berman declaration does not state that exsanguinations does not affect expression as assayed in live versus dead mammals or provide the efficacy of taking a biopsy of the cerebral cortex of a human.

First, as stated by Dr. Berman in the Berman declaration at ¶ 7, the post-exsanguination collections performed for the studies of the instant application were performed carefully to ensure reliable results. Also, the specification as originally filed at paragraph [0098] shows that proper procedures were conducted to ensure reliability. Additionally, one of ordinary skill in the art would have recognized that this method is

accepted in the art (also as stated in the Berman declaration at ¶ 7). Indeed, this is supported by Roberts in that necropsy in Roberts was performed on the macaques with subsequent intracardiac perfusion. Therefore, this method was an accepted method to one of ordinary skill in the art to report on gene expression in live mammals, even though the animals were sacrificed and gene expression was determined from the sacrificed animals. Finally, the Applicants previously demonstrated that the use of humans in research studies to develop assays are many times over-invasive and unjustified (Berman declaration at ¶ 9). However, once the assay has been developed, the assay may then be justified for use in humans to provide reliable indication of a disease-state. Indeed, such an assay may be used to help confirm a diagnosis based on other factors, such as cognitive symptoms for NeuroAIDS. As shown below, the use of the macaque animal model in determining upregulation of macaque Cripto-1 in macaque NeuroAIDS as described in the instant application reliably reports that human Cripto-1 (SEQ ID NO: 1) is upregulated in human NeuroAIDS. Therefore, upregulation of Cripto-1 (SEQ ID NO: 1) in a human cerebral cortex biopsy would indicate NeuroAIDS in that human.

(iii) Detection of Macaque Homologue/SEQ ID NO: 1, (iv) Human Studies, and (v) Macaque Monkey Model for Humans

The Examiner continues to assert that the specification as filed does not teach detection of SEQ ID NO: 1 but the macaque homologue of SEQ ID NO: 1. The Examiner also states that the claims are not drawn to methods of studying NeuroAIDS but detecting NeuroAIDS and thus arguments about the importance of animal models are beyond the scope of the claimed invention. The Examiner also states that there were no studies performed in humans and that the macaque monkey is not a reasonable model for humans.

As stated above, enablement relates to what one of ordinary skill in the art would have recognized as acceptable methods to detect NeuroAIDS. Thus, although the studies described in the specification as filed identified the macaque homologue as upregulated in macaque NeuroAIDS, if one of ordinary skill in the art would have recognized that this was an acceptable method to indicate that SEQ ID NO: 1 is upregulated in human NeuroAIDS, then the method as claimed is enabled. Therefore, the previously made arguments concerning the importance of animal models are indeed relevant in that, as stated before and

as further detailed below, the animal model methods used as described in the specification as filed were the art-accepted methods of reporting on human NeuroAIDS.

One of ordinary skill in the art would have recognized that the use of the macaque animal model in determining upregulation of macaque Cripto-1 in macaque NeuroAIDS as described in the instant application reliably reports that human Cripto-1 (SEQ ID NO: 1) is upregulated in human NeuroAIDS. First, as stated throughout the Berman declaration (*e.g.*, ¶¶ 10, 12, and 13), the use of a macaque animal model to study NeuroAIDS was accepted by those of ordinary skill in the art to report on NeuroAIDS in humans. Also, Roberts (which was cited by the Examiner) supported the use of macaque monkey models of NeuroAIDS to report on human NeuroAIDS at the time of filing of the instant application (2003). For example, the abstract and final paragraph of the Discussion section (on page 2055) go back and forth in discussing the results obtained in terms of macaque NeuroAIDS and human NeuroAIDS. (However, as discussed above, the Roberts study did not go far enough in confirming their results as cautioned by Chismar, something the Applicants did do.) Furthermore, Chismar, which was published by the Roberts laboratory, states, "The study of gene expression in nonhuman primates represents a critical area of research directly related to the understanding of human health and physiology. Nonhuman primate studies play a crucial role in organ transplantation, vaccine development, viral pathogenesis, gene therapy, and a host of other human health related technologies." (Chismar at page 516, first and second column, bridging paragraph.) Chismar also states that in order to realize the benefits of using these studies, the use of human primers to identify the nonhuman primate homologue (to report on the human disease) was a solution in the pertinent art to the problem of not having fully sequenced monkey genomes.

> While the use of gene expression microarrays in nonhuman primate models will clearly advance our understanding of human health and disease, the availability of adequate sequence data keeps this technology out of reach in the near term. One potentially powerful short-term solution to this problem is the use of cross-species hybridization: human sequence-based microarrays used to analyze nonhuman primate genomics.

(Chismar at page 516, second column, first full paragraph.) Additionally, the Berman declaration at ¶ 8 states how the study of the instant application used human primers to

identify upregulation of macaque Cripto-1 in macaque NeuroAIDS and that this indicates upregulation of human Cripto-1 (SEQ ID NO: 1) in human NeuroAIDS.

Therefore, one of ordinary skill in the art would have recognized at the time of Chismar (published in 2002) and the filing of the instant application (priority date of 2003) that (1) the use of a macaque model of NeuroAIDS was a method accepted by ordinary artisans to report on conditions in human NeuroAIDS, (2) the use of human primers to determine upregulation of macaque genes was a method accepted by ordinary artisans, and (3) the upregulation of macaque genes in the macaque NeuroAIDS model as indicated by human primers would provide a method accepted by ordinary artisans to indicate upregulation of human Cripto-1 (SEQ ID NO: 1) in human NeuroAIDS.

(v) SHIV Infection as a Model for Human NeuroAIDS

The Examiner continues to allege that the SHIV infection used in the instant application was not a reasonable model for human NeuroAIDS. The Examiner alleges that Williams et al., J. Neurovirol., 14:292-300 (2008) ("Williams") shows differences in human and animal NeuroAIDS models, that Buch and Sui show evidence of unpredictability, and that Stephens questions whether overexpression of Cripto-1/SEQ ID NO: 1 is diagnostic of NeuroAIDS.

SHIV infection of macaque was accepted by ordinary artisans as a model for human NeuroAIDS. First, as discussed above, macaque models of NeuroAIDS were acceptable to those of ordinary skill in the art to indicate conditions of human NeuroAIDS. Further, SHIV infection of macaques was recognized as an acceptable method to produce NeuroAIDS in macaque to provide an art accepted model of human NeuroAIDS. This was shown previously by the Applicants in the Berman declaration (*e.g.*, at ¶¶ 12 and 13) and in the previous response. The Examiner, however, tries to show where Williams, Buch, and Sui highlight differences among SIV and SHIV.

The Applicants agree that there are differences in SIV and SHIV pathologies. However, the Applicants used SHIV, not SIV; and as detailed in the previous response, SHIV is superior to SIV in many regards. First, Williams states, "Such innovation [development of pathogenic SHIV by passaging] has led to the development of several strains and variations

of SHIV, each one with specific characteristics in regard to pathogenic phenotype that make it convenient for studying certain aspects of AIDS" (Williams at page 295, first and second columns, bridging paragraph); "SHIV infection in macaques creates a model system closely resembling the disease course of human AIDS" (Williams at page 295, second column, first full paragraph); and

> Another key feature of the SHIV/macaque model is that the pathology of certain tissues and organ systems, such as the CNS, are compatible with the corresponding pathological changes seen in human AIDS. This underscores the utility of SHIV in examining specific HIV-1-induced disease pathology, such as encephalitis, making SHIV an essential model for the study of neuro AIDS

(Williams at page 295, first and second columns, bridging paragraph, citations omitted). Thus, SHIV in macaques closely resembles HIV in humans. In the most recent Office Action, the Examiner specifically cited several passages, including the following passage at page 16: "Although SIV-infected macaques have been critical in our understanding of lentiviral neuropathogenesis, the genetic relatedness of these viruses in more to HIV-2 than HIV-1, especially in the *env* gene, which raises the question of whether these models accurately mimic the neuropathogenesis of HIV-1 infection." (Williams at *Id.*) However, this passage was cited by the Applicants in the previous response, including the sentence immediately following it: "The construction of the chimeric of SHIV has led to significant advances in the field of AIDS, particularly in the study of immune response and vaccine development." (Williams at *Id.*) Therefore, contrary to the Examiner's assertions, Williams supports the Applicants' position that SHIV infection of macaque was recognized by one of ordinary skill in the art as an acceptable model of human NeuroAIDS and, indeed, is stated to be a better model than SIV infected macaque.

A closer analysis of Buch also reveals that this reference supports the Applicants and not the Examiner. Buch does state that HIV and SHIV use different receptors for infection, CCR5 (R5) and CXCR4 (X4) receptors, respectively, as pointed out by the Examiner. However, again, one of ordinary skill in the art recognized that, even with these differences, macaque/SHIV was the best model system (as discussed above), and this system was recognized by ordinary artisans as indicative of human NeuroAIDS (*e.g.*, *see* the Berman declaration at ¶ 10). Furthermore, although viruses such as SHIV that use CXCR4 as the

receptor are stated in Buch as not having any known role in late pathogenesis of human HIV (Buch at page 72, first and second columns, bridging paragraph), Buch also states that

> Gorry et al. (2001) [J. Virol., 75:10073-10089] later showed that although R5 viruses are usually associated with neuropathological effects of the infection, macrophage-tropic X4 viruses can have a similar role because this viral phenotype was identified in brain sections with lesions characteristic of the neuropathological effects of HIV infection. Since macrophages are the main viral target cell in the brain, the inference was that macrophage-tropic X4 HIV could be neuropathogenic. Our studies on X4 $SHIV_{KU2}$ infection in macaques lent support of this concept.

(Buch at *Id.*). Therefore, one of ordinary skill in the art would have recognized that infection by X4 viruses such as SHIV were indicative of pathologies in human NeuroAIDS.

Stephens does state, as noted by the Examiner, "Whether cripto-1 expression is elevated during the course of neuroAIDS due to enhanced expression of one or more cytokines/chemokines remains to be determined." (Stephens at pages 97-98, bridging paragraph, final sentence.) Again, as stated above, enablement relates to what one of ordinary skill in the art would have accepted. The Examiner appears to suggest that Cripto-1 upregulation may be observed with abnormalities in cytokine/chemokine expression independent of HIV/SHIV infection. However, Sui states, "Human immunodeficiency virus (HIV)-encephalitis results from a cascade of viral-host interactions that lead to cytokine and chemokine imbalance, which then leads to neuropathologic manifestations of the disease." (Sui at first sentence of the abstract). Thus, the manifestations of human NeuroAIDS itself is caused by cytokine/chemokine imbalance. Also, just as discussed above how ordinary artisans did not infect controls with any virus at all, one of ordinary skill in the art would have recognized that regardless of the identification of the identity of the molecule(s) that directly allow(s) for/produce(s) upregulation of Cripto-1, the upregulation of Cripto-1 indicates the presence of NeuroAIDS. As a simple analogy, a high level of blood glucose is indicative of diabetes; however, whether that diabetes is Type I (failure to produce insulin) or Type 2 (insulin resistance) can not be determined simply by measuring the blood glucose levels, even though a diagnosis of diabetes may still be made. Here, although it is not known what molecules interact to cause upregulation of Cripto-1 in NeuroAIDS, Cripto-1 is still indeed upregulated during NeuroAIDS. Therefore, one of ordinary skill in the art would have recognized that Cripto-1 upregulation is indicative of NeuroAIDS, even if the underlying mechanism was not known.

(vi) Nexus between SEQ ID NO: 1 and NeuroAIDS

The Examiner has alleged that there is no nexus for the role of upregulated SEQ ID NO: 1 and NeuroAIDS. Specifically, the Examiner alleges that one of ordinary skill in the art would have to determine if 2.5-fold overexpression is predictive of NeuroAIDS in view of the small sample size used in the specification and in view of variation among individuals (citing Cheung). The Examiner also alleges that upregulation of SEQ ID NO: 1 is not indicative of a disease state since it is a homologue of the macaque Cripto-1 upregulation described in the instant specification.

In regards to determining what level of overexpression indicates a disease state, again, it is what one of ordinary skill in the art would have recognized as indicating the disease state. One of ordinary skill in the art would have recognized that the sample size used in the instant specification was acceptable, as detailed above. Further, as discussed in detail below, one of ordinary skill in the art would have recognized that the variations among individuals and species exist but that the studies used in the instant specification were acceptable to report on conditions of human NeuroAIDS generally.

The 2.5-fold difference, as indicated by the specification as filed at ¶ [0073], was an arbitrary cut-off value for the microarray experiments. However, the exact level of overexpression of Cripto-1 need not be claimed. As discussed above, the Applicants performed RT-PCR experiments which showed that Cripto-1 was indeed upregulated, confirming the results of the microarray experiments. Therefore, although the 2.5-fold difference was selected as arbitrary, the upregulation of Cripto-1 in an art accepted model of human NeuroAIDS observed in the microarray experiments were not false positives but true positives and therefore demonstrate that upregulation of Cripto-1 beyond this level is a reliable indicator of NeuroAIDS.

Finally, as discussed above, one of ordinary skill in the art would have recognized that the macaque/SHIV model used in the instant application was an acceptable model of human NeuroAIDS. Therefore, the upregulation of macaque Cripto-1 in macaque NeuroAIDS would reliably report on the upregulation of human Cripto-1 (SEQ ID NO: 1) in human NeuroAIDS.

(vii) SHIV in Two Members of the Macaque Family; SIV- and SHIV-Induced NeuroAIDS Pathologies

The Examiner continues to allege that Raghaven teaches SHIV has different affects on two members of the macaque family and that SIV- and SHIV-induced NeuroAIDS have different pathologies. The Examiner also cites Buch as citing Raghaven and stating that rhesus and not pigtailed macaque developed neurological disease in $SHIV_{KU2}$ infected animals.

Applicants reiterate (*see* the Berman declaration at ¶12) that the virus used in the studies of the instant application was $SHIV_{50OLNV}$ (*see* the specification as filed, *e.g.*, at ¶ [0075]), not $SHIV_{KU2}$ (used in Raghaven (Raghaven at page 852, second column, first full paragraph) and discussed in Buch) or SIV. As stated above, SHIV is a better virus than SIV in producing a human model of NeuroAIDS. Also, the $SHIV_{50OLNV}$ strain used in the instant application was shown to be activated in pig-tailed macaques (*see* Example 1 of the instant application). The differences seen in Raghaven are due to activation of the virus in rhesus macaque versus pig-tailed macaque. (Raghaven at page 856, second column, first full paragraph). Furthermore, Raghaven is silent as to whether the activation or non-activation of SHIV alters Cripto-1 expression. As stated in the previous response, non-activation of SHIV in pig-tailed macaque as described in Raghaven does not in itself foreclose the possibility of upregulation of Cripto-1. Indeed, the Applicants used a different viral strain that *was* activated in pig-tailed macaque and produces a better model of human NeuroAIDS due to this activation, which showed upregulation of Cripto-1. Therefore, one of ordinary skill in the art would have recognized that the methods as used by the Applicants would reliably report on upregulation of Cripto-1/SEQ ID NO: 1 in human NeuroAIDS.

(viii) Intra- and Interspecies Variation

The Examiner continues to allege that intra- and interspecies variations make the use of upregulation of Cripto-1/SEQ ID NO: 1 unpredictable for indicating NeuroAIDS. The Examiner contends that the claims are overly broad as being directed to any mammal and that the location of the sample affects gene expression patterns. The Examiner has cited Cheung, Wu, and Enard and additional, newly cited articles (Benner et al., Trends in Genetics, 17:414-418 (2001) ("Benner"); May et al., Science, 241:1441-1449 (1988) ("May"); and Caceres et

al., PNAS, 100:13030-13035 (2003) ("Caceres")) and states that the macaque used were outbred.

In order to advance prosecution, the claims have been amended to recite that the mammal is macaque or human. Therefore, the claims are not directed to "any" mammal. As discussed throughout this response, the macaque NeuroAIDS model used by the Applicants reliably reports on conditions of human NeuroAIDS.

Roberts does state that the proximity of neurons to the source of damaging molecules is a key factor in neuronal pathogenesis (Roberts at page 2055, second column, first full paragraph), as cited by the Examiner. However, this argument is of no moment in that the claims recite that upregulation of SEQ ID NO: 1 indicates NeuroAIDS. The claims state nothing regarding normal expression of or downregulation of SEQ ID NO: 1 or what this would indicate. There would have been no undue experimentation in determining whether SEQ ID NO: 1 is upregulated, as discussed throughout this response, and if SEQ ID NO: 1 is upregulated, this indicates NeuroAIDS.

Regarding variations, as stated throughout this response, again, enablement relates to what one of ordinary skill in the art would have known at the time of filing the patent application. As stated in the Berman declaration at ¶ 13, at the time of filing the instant application, one of ordinary skill in the pertinent art of studying human NeuroAIDS would have recognized that the methods used by the Applicants were accepted methods, even though intra- and interspecies variations exist. It is recognized generally that such variations exist, as provided in Cheung, Wu, Enard, Benner, May, and Caceres. However, one of ordinary skill in the art of human NeuroAIDS would have recognized that there are art accepted methods of compensating for these variations to provide animal models that reliably report on disease conditions in humans. The Applicants recognized that macaques are outbred and compensated by using a more stringent cut-off for the microarray experiments (specification as filed at ¶ [0073],), later confirmed by RT-PCR, as discussed above. Indeed, Roberts, as cited by the Examiner and as discussed above, uses a macaque NeuroAIDS model to report on conditions in human NeuroAIDS. Additionally, Chismar, published by the same laboratory as Roberts and discussed above, addresses the variation inherent in the methods as used by the Applicants and concludes that the approach is acceptable (Chismar at abstract), of

course with the caution that validation should be done (as the Applicants did do, as discussed above). Also, Chismar cites Enard (Chismar ref. no. 3). Therefore, Chismar, recognizing the variations as taught by other fields, teaches how one of ordinary skill in the human NeuroAIDS art would compensate for the variations to produce an art acceptable animal model to report on human NeuroAIDS. Again, it is what one of ordinary skill in the pertinent art would have recognized as enabling. Here, as evidenced by Chismar, the Applicants did what was accepted in the art to show that SEQ ID NO: 1 is upregulated in human NeuroAIDS.

For the foregoing reasons, the present application enables methods of detecting NeuroAIDS in assaying the expression level of Cripto-1/SEQ ID NO: 1. Withdrawal of this rejection is respectfully requested.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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